Isolation Of Bacteria From Coal Mine Dust With Metal Nanoparticle Fabrication Ability

A THESIS SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

Master of Science

In

Life science

By

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413LS2044

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ROURKELA-769008,

Odisha,India, 2015

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CERTIFICATE

This is to certify that the thesis entitled "Isolation Of Bacteria From Coal Mine Dust With Metal Nanoparticle Fabrication Ability" submitted by Subhasmita Panda, roll no.413LS2044 for the award of Master of Science degree from National Institute of Technology, Rourkela is a record of bonafide work, carried out by her under my supervision. Results embodied in this thesis serve to be new and has not been submitted to any university for award of any degree or diploma.

10/5/15

DR. SUMAN JHA

DECLARATION

To the best of my knowledge the research project report entitled ‘‘**Isolation Of Bacteria From Coal Mine Dust With Metal Nanoparticle Fabrication Ability’’** reported here in its original and has been submitted to National Institute of Technology, Rourkela for partial fulfilment of the degree of Master of Science in Life Science is a bonafide record of the project work carried out by me under the supervision of Dr. Suman Jha, Assistant Professor, Department of Life Science, National Institute of Technology, Rourkela. This thesis is my own work and that, to the best of my knowledge and belief, the matter and results of this thesis has not been submitted by any other research persons or any students.

I do hope this project work will satisfy our beloved teachers. I solicit kind and favourable appreciation.

Ms. SUBHASMITA PANDA

ACKNOWLEDGEMENT

First and foremost I bow down before the almighty God who has made everything possible.

It is a great pleasure and proud privilege to express my deep sense of gratitude and everlasting indebtedness to my research supervisor, **Dr. Suman Jha,** Assistant Professor, Department of Life Science, NIT, Rourkela. I am grateful to him for providing me substantial knowledge, incisive guidance, helpful advices and moral support all the time during my project work. I would like to thank him for patiently scrutinizing the preparation of this project report and making my work a successful one.

I would like to express my sincere thanks to Dr. Sujit Bhutia and all the faculty members and staffs of Department of Life Science for their constant support and encouragement throughout my M.sc. years.

I am highly obliged to my mentor Mr. Manoranjan Arakha for his patience, immense support and untiring supervision in the experimental works, interactive discussions and motivational encouragements in successfully carrying out this piece of work. My special thanks to Mr Parth Sarthi Nayak, Ms. Shreyasi Asthana and Ms. Kanti kusum Yadav (PhD Scholars of Protein Biology Lab) Department of Life Science, National Institute of Technology, Rourkela for their timely help, good wishes and moral support during my dissertation. I would also like to thank all my classmates and my lab mates for their help and support during my work.

Last but not the least, I would not have been able to complete this project without the love and support of my parents whose immense faith in my abilities helped me to overcome many obstacles and march ahead during all the difficult times. I would like to dedicate this project to my beloved parents and my sisters.

Subhasmita panda

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ABBREVIATION

NPs: Nanoparticles

Ag-Silver

(AgNO3)-Silver nitrate

DLS-Dynamic light scattering

ATR-FTIR-Fourier transform infrared spectroscopy

nm-nanometer

MIC-Minimum inhibitory concentration

mV-mili volt

FE-SEM-Field Emission Scanning electron microscope

Hrs-Hours

Mins-Minutes

rpm-Rotation per minute

ABSTRACT

Nanoparticles being the smallest unit of nanotechnology are playing important roles in various fields. There are different kinds of nanoparticles ranging from metals to non-metals, and also from carbon sources. Though various methods have been optimized for synthesis of nanoparticles, still biological methods for synthesis of metal and metal oxide nanoparticles have drawn attention of different research groups due to their advantageous properties over physical and chemical methods. The objective of this study is to synthesize silver nanoparticles using bacteria isolated from coal mine. These bacteria are chosen assuming that they have beter quality for metal tolerance than normal bacteria. The silver nanoparticles are synthesized at the MIC value of $AgNO_3$ against the isolated bacteria. The characterization of nanoparticles samples were done using different techniques like UV-Vis spectroscopy, DLS, FE-SEM, and ATR-FTIR proved that they are silver nanoparticles with coating of different bacterial moieties like proteins, carbohydrates, etc.

1. INTRODUCTION

Bacteria are microscopic, relatively simple, prokaryotic organisms whose cells lack a nucleus. They are typically in few micrometer in length. They are in different shape ranging from spheres to rods and spirals. Different bacteria are present in different area such as in soil, acidic, hotspring, mine area, water etc. Bacteria are found everywhere in the ocean. They are not distributed uniformly. The bacteria are a part of marine plankton. Most bacteria are present in the sunlit upper layer and their number decrease with depth. The most well known marine bacteria are the other culturable group, the cyanobacteria. Two major groups of cyanobacteria can be found in the world ocean. The common and natural component of lakes, rivers, and streams are bacteria.Some are may be harmless to humans and normally inhabit the intestinal tract of warm-blooded animals.The bacteria also cause sickness and disease in humans. All thermophiles require a hot water environment. Thermophile bacteria are heat-loving.They grow in high temperature of 50°C or more. A billion bacteria are present in a gram of soil.The soil bacteria can be classified as three groups Cocci (round/spherical), rod shaped and spirilla . Bacili are most numerous followed by cocci and spirilla in soil. The bacteria in soil brings a number of changes and biochemical transformation. The soil bacteria play a vital role in nitrification, ammonification, denitrification, biologica fixation of atompsoheric nitrogen (symbiotic and nonsymbiotic), oxidation and reduction of sulphur and iron compound. The soil bacteria also play a significant role in plant nitrution. In coal mine area thirty-four forms of bacteria are isolated. Mostly they are in similar form. Ten of these forms are pigment formers belonging to the genera *Pseudomonas, Sarcina and rhodococcus*. The bacteria are existed in very early time, probably long before the carboniferous period.

Bacteria are capable of mobilization and immobilization of metals.Some bacteria reduce metal ions to preceipitate metals at nanometer scale. Biosynthesis of nanoparticle using bacteria has emerged as rapidly developing research area in green nanotechnology (Iravani)]. In this 21st century of science Nanotechnology has a broadened field for developing molecules or objects in the nano range i.e, 1 to 100nm. Nanotechnology has a vital role in food, cosmetics, health care, biomedical services, space industries, gene delivery, mechanics, optics and photoelectrochemical application. Nanoparticles are ultrafine particles having one of its dimension in the range of 1-100 nm. The high mobility in free state, enormous specific surface area and the tendency to express quantum effects are the silent features of nanoparticle. On the basis of characteristic features a nanoparticle can be differentiated accordingly. Some are positively charged, some are negatively while others are neutral. Nanoparticles may be either magnetic or nonmagnetic types and they can also classified organic (as carbon) and inorganic (silver nanoparticles, gold nanoparticles). Nanoparticle can be classified into five categories:

- Fullerenes and Carbon Nanotubes
- Metals
- Ceramics
- Semiconductors (Quantum dots)
- Polymeric

Physical and chemical properties of nanoparticles that may change at the nanoscale like

- Color
- Melting temperature
- Crystal structure
- Chemical reactivity
- Electrical conductivity
- Magnetism

- Mechanical strength

The nanoparticles can be synthesized using physical method which is known as top-down approach.The top-down approach deals with methods such as thermal decomposition, diffusion, irridation, arc discharge etc. The chemical and biological method which known as bottom up approach .The chemical method involves electro chemical synthesis, chemical reduction etc. The chemical agents such as N,N-dimethyl formaide(DMF), poly,ethyl alcohol,tetra-n-tetra fluoroborate are also used in synthesis of nanoparticle. Mainly microbes and plants are also used as biological agent. The synthesis of nanoparticles in biological way is also known as “Green synthesis”. Biological methods for synthesis of nanoparticle employ the use of biological agents like bacteria, fungi, yeast, actinomyces and plants. Biological agents secrete a large amount of enzymes which are capable of hydrolysing metals and thus bring about the enzymatic reduction of metal ion (Kashyap, Kumar et al.) .

Silver has the highest electrical and thermal conductivity among metals, making it a popular material for different applications. Silver nanoparticle have unique optical, thermal and electrical properties. Different techniques such as transmission and electron microscopy (TEM, SEM), atomic force microscopy (AFM), dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), powder X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), and UV–Vis spectroscopy are used for the characterization of silver nanoparticle (El-Nour, Eftaiha et al.). The Silver nanoparticle used to know the antifungal effects on fungi tested with low haemolytic effects against human erythrocytes. Silver nanoparticle has antimicrobial activity. Biologically synthesized silver nanoprticles have many applications including in textile industry.

2.LITERATURE REVIEW

2.1. Introduction to nanotechnology

Nanotechnology is defined as a highly developing field because of its vast array of applications in various fields of medical science, technology and various research areas. The meaning of the word ‘Nano’ is extremely small or dwarf which originated from a Greek word. The term Nanotechnology was later coined by Professor Norio Taniguchi by using Feynman’s explorations of ultra precision machining in 1974 as follows: “Nano-technology mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or by one molecule”. The first use of ‘nanotechnology was in “There’s Plenty of Room at the Bottom” a talk given by physicist Richard Feynman at an American Physical Society meeting at Caltech on December 29, 1959.

The study about nanoscience and nanotechnology provides the well developed applications of exceptionally miniature things and be capable of the encroachment of all the fields of scientific research and development like physics, chemistry, materials and metallurgy engineering, biology and also in biotechnology(Rai, Yadav et al. ; Huang, Li et al. 2007; Mude, Ingle et al. 2009) . The nanoparticle interaction with biological materials lead to the formation of new nanomaterial with control size, shape, surface chemistry, roughness and surface coatings.(Singh, Shukla et al.). The term “nanoparticles” is used to describe a particle with size in the range of 1nm-100nm, at least in one of the three possible dimensions. In this size range, the physical, chemical and biological properties of the nanoparticles changes in fundamental ways from the properties of both individual atoms/molecules and of the corresponding bulk materials. Nanoparticles can be made of materials of diverse chemical nature, the most common being metals, metal oxides, silicates, non-oxide ceramics, polymers.

2.2:Classification of nanoparticles

Nanoparticles mainly classified as: organic(fullerenes) and inorganic(magnetic nanoparticles, noble metal nanoparticles like gold and silver) nanoparticles.The semiconductor nanoparticles are like titanium dioxide and zinc oxide).The inorganic nanoparticle provide superior material properties with functional versatility .So they are known as noble metal nanoparticles (gold and silver). The inorganic nanoparticle are available in chemical imaging drug agents and drugs due to their special features and advantages. The inorganic particles have been examined as potential tools for medical imaging as well as for treating diseases. Inorganic nonmaterial have been widely used for cellular delivery due to their versatile features like wide availability, rich functionality, good compatibility, and capability of targeted drug delivery and controlled release of drugs (Xu, Zeng et al. 2006).

2.3:-Silver nanoparticle

The synthesis of nanoparticle occurred either bottom-up or from top-down approach. The unique properties of silver nanoparticle (e.g., size, shape, optical, electrical, and magnetic properties). Due to the unique character of nanoparticles, they can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing silver nanoparticles. Silver nanoparticles have been successfully synthesized from Gram negative bacteria like *E.coli*. Silver nanoparticles have a characteristic feature of absorbing light at a wavelength of 420 nm (Pal, Tak et al. 2007).

2.4:Application of nanoparticle

Due to their extremely small size and large surface to volume ratio, nanoparticle lead to both chemical and physical differences in their properties compared to bulk of the same chemical composition. The important properties of nanoparticles are mechanical, biological and sterical properties, catalytic activity, thermal and electrical conductivity, optical absorption and melting point etc (Mei, Oh et al. 2009). Therefore, designing and production of materials with novel applications can be resulted by controlling shape and size at nanometer scale. These particles also play an important role in medical imaging, nano-composites, filters, drug delivery, and hyperthermia of tumors (Panigrahi ; Tripathy, Raichur et al. ; Tan, Wang et al. 2006). Solar steam light is a successful invention of nanotechnology. Due to the scarcity of electricity which is useful in case of the wide application of water purification and disinfecting in the dental instruments. Silver nanoparticles have drawn the attention of researchers because of their extensive applications in areas such as integrated circuits, sensors, biolabelling, filters, antimicrobial deodorant fibres (Panigrahi ; Huang, Li et al. 2007), cell electrodes (Mandal, Bolander et al. 2006), low-cost paper batteries (silver nano-wires)(Hong, Park et al. 2006), and antimicrobials(Cho, Park et al. 2005; DurÃ¡n, Marcato et al. 2007), Silver nanoparticles have been used extensively as antimicrobial agents in health industry, food storage textile coatings and a number of environmental applications. Antimicrobial properties of silver nanoparticles caused the use of these nano-metals in different fields of medicine, various industries, animal husbandry, packaging, accessories cosmetics, health and military. For instance, it was shown that silver nanoparticles mainly in the range of 1-10 nm attached to the surface of *E. coli* cell membrane.(Hong, Park et al. 2006). Silver nanoparticle also used in pharmaceutics, medicine, and dentistry. In Pharmaceutics & medicines, silver nanoparticle are used in treatment of dermatitis; inhibition of HIV-1, treatment of ulcerative colitis & acne, as antimicrobial agents against infectious organisms, remote laser light-induced opening of microcapsules, silver/dendrimer nanocomposite for cell labeling, molecular imaging of cancer cells, enhanced Raman Scattering (SERS) spectroscopy, detection of viral structures (SERS & Silvernanorods), coating of hospital textile (surgical gowns, facemask), additive in bone cement, implantable material using clay-layers with starchstabilized AgNPs, orthopedic stocking, hydrogel for wound dressing. In dentistry silver nanoparticle are used in additive in polymerizable dental materials patient, silver-loaded SiO_2 nanocomposite resin filler (dental resin composite), polyethylene tubes filled with fibrin sponge embedded with AgNPs dispersion.

2.5: Methods for nanoparticle synthesis

Nanoparticle can be synthesized by top-down and bottom-up approach. Nanoparticles can be synthesized with the help of physical, chemical and biological processes.

Physical and chemical methods of nanoparticle synthesis

Some of the commonly used physical and chemical methods include:

- Sol-gel technique, is used in chemical method .It is a wet chemical technique which is used for the fabrication of metal oxides from a chemical solution. For integrated network (gel) of discrete particles or polymers it is used as precursor. It can be either deposited on the substrate to form a film, cast into a suitable container with desired shape or used to synthesize powders.
- The polar solvents under pressure and at temperatures above their boiling points are used in solvothermal synthesis. The solubility of reactants increases significantly under solvothermal conditions, enabling reaction to take place at lower temperature.

- The reduction of an ionic salt in an appropriate medium is done in chemical reduction method.It ocuur in the presence of surfactant using reducing agents. Sodium borohydride, hydrazine hydrate and sodium citrate are commonly used reducing agent.
- Laser ablation, in which removing material ocuur from a solid surface by irradiating with a laser beam is . The material is heated by absorbed laser energy and evaporates or sublimates at lower laser flux.The material is converted to plasma at higher fiux.The depth over which laser energy is absorbed and the amount of material removed by single laser pulse depends on the material’s optical properties and the laser wavelength determined the depth over which laser energy is absorbed and the amount of material removed by single laser pulse . This method also produced Carbon nanotubes.
- Inert gas condensation, in which different metals are evaporated in separate crucibles.It ocuur inside an ultra-high vacuum chamber which filled with helium or argon gas at typical pressure of few100 pascals. As a result, the evaporated metal atoms lose their kinetic energy and condense in the form of small crystals.They accumulate on liquid nitrogen filled cold finger. e.g. gold nanoparticles are synthesized from gold wires.

Biosynthesis of nanoparticles

The biosynthesis of nanoparticles is needed because it is a cheaper pathway than physical and chemical.In this synthesis microorganisms and plant extracts used. For the synthesis of nano and micro length scaled inorganic materials nanoparticles nature devised various process (Mohanpuria, Rana et al. 2008).This process used both extracellular and intracellular method for the synthesis of nanoparticles . In intracellular method, the bacterial cell filtrate is treated with metal salt solution and kept in a shaker in dark at ambient temperature. In extracellular method , *aspergillus niger* was used.The cellwall of microorganism play a major role in the intracellular synthesis of nanoparticle.

2.6: Why green synthesis?

Green nanotechnology encourages not only fundamental but also goal-oriented research in both the academic and industrial fields for the design and development of green nanoparticles (GNPs). Green nanoparticles have already been used in the design of smart electronic devices, life-saving nano-pharmaceuticals, and in substitute of green energy production devices as well. In the search of cheaper pathways for nanoparticles synthesis, scientist used microbial enzymes and plant extracts (phytochemicals). With their antioxidant or reducing properties they are usually responsible for the reduction of metal compounds into their respective nanoparticles. Green synthesis is cheaper than chemical and physical methods as they are cost effective, environment friendly, easily scaled up for large scale synthesis and in these methods there is no need to use high pressure, energy, temperature and toxic chemicals.

Objectives

1. **Isolation of bacteria from coal mine and evaluation of $AgNO_3$ MIC against these bacteria**
2. **Synthesis of silver nanoparticles using these bacteria**
3. **Characterization of silver nanoparticles using UV-Vis, zeta analyser, FE-SEM, and ATR-FTIR spectroscope.**

3. MATERIALS AND METHODS

3.1.Materials

3.1.1 Chemicals required

Nutrient broth, nutrient agar, glutaraldehyde, tannic acid, crystal violet, iodine complex, safrannin, decolarizer were purchased from Himedia, India. Silver nitrate ($AgNO_3$), was purchased from Sigma, India.

3.1.2 Glass wares

All glass wares used (conical flask, beaker, measuring cylinder, test tubes, petriplates) were purchased from Borosil, India.

3.1.3 Sample collection

The bacteria used for synthesis of silver nanoparticles was isolated from the soil samples collected from the Sambleshwari open crust project, Lajkura, district Jharsuguda, Odisha, India.

Fig:1:- Sample collected site of Lajkura coal mine

3.2: METHODS

3.2.1: Isolation of bacterial samples

For isolation of bacterial strains, 0.1g coal powder was measured and dissolved in 1ml of distilled water. 20 µL of bacterial samples were spread on the agar plates after 1000 dilutions following the principles of serial dilution. The plates were kept overnight incubation at 37 ^{o}C. The slant cultures of bacterial strains were prepared for further use by taking single colonies from the plates.

3.2.2: Gram staining

Gram staining was performed to check whether the bacteria were Gram +ve or Gram -ve. For the Gram staining, first the bacteria strain from mother culture was fixed in the slide. The crystal violet was flooded for 30sec over the slide. The slide was rinsed with H_2O and drained the slide against a paper towel. Iodine was added for 1min. Again it was rinsed with H_2O and drained carefully. It was washed with Gram decolorizer (ethanol) for 30seconds. Again it was rinsed with H_2O and saffranine was added to it for 1min, followed by observing the slide under the light microscope.

3.2.3:Determination of MIC of $AgNO_3$

MIC was determined to check the minimum concentration of silver metal ions at which the growth of bacteria can be inhibited. It is important to know the tolerance of bacteria against silver metal ions. The mother culture of two bacteria was prepared by inoculating a loop full of bacteria into 5ml nutrient broth and incubated at 150rpm and 37 ^{o}C for 16hrs. Different reaction mixtures were prepared in 96 well plate taking different concentrations of $AgNO_3$ and 20 mL of bacterial culture, and volume was adjusted to 300 ml with nutrient broth, and

O.D. was taken at 300 nm using plate reader at 37 ^{o}C. CFU measurement was performed to estiment the number of viable bacteria cells in the sample. The Samples were taken from different wells like control, 0.019mM, 0.15mM, 1.25 mM and spread on nutrient agar plate after 10000 times dilutions..The plates were kept overnight in incubator at 37 ^{o}C for their proper growth, colonies were counted after 24 hrs of incubation.

3.2.5: Synthesis and purification of $AgNO_3$

For synthesis of AGNPs, 5 mL mother culture was done in nutrient broth and kept in incubator at 37 ^{o}C and 150 rpm. After 24hrs 1 mL of this culture was added to 100 mL of nutrient broth and appropriate amount of $AgNO_3$ was added so as to obtain the MIC value (0.15 mM). The reaction mixture was kept for further 24 hrs for nanoparticles synthesis. The bacterial culture containing the nanoparticles were sonicated followed by centrifugation at 6000 rpm. The supernatant was collected for further analysis.

3.2.6:Characterization of AgNP

(A) UV-Vis spectroscopic analysis

To check the surface plasmon resonance property of nanoparticle synthesized from bacteria it is necessary to go for its UV-Visible spectrum analysis which reveals the specific type of nanoparticle absorbing a specific wavelength of light. This property can distinguish silver nanoparticles from others and can also state whether it is silver or not present in the solution. UV-Visible spectroscopy works on the principle of light absorption depending on the concentration of particles in the solution. Silver nanoparticle has a unique property of surface plasmon resonance. Here the electron on the metal surface has its own frequency due to oscillation against the electro positive nuclei. In the case of nanoprticles SPR is known as localized surface Plasmon resonance.

(B) Dynamic light scattering analysis

The size distribution of particles in solution is determine in the Dynamic light scattering . Particles of different sizes can scatter light to different angles on the basis of their difference in size. The scattering of light is based on particle size, smaller particles can scatter light strongly while increase in particle size leads to less scattering. Samples were analyzed after synthesis of nanoparticles.

(C) Zeta potential analysis

It was used to measure the potential difference between the dispersed medium and the stationary layer of fluid attached to the dispersed particles and determines the net charge on the surface of nanoparticle. For zeta potential analysis the sample was diluted in deionized water and filtered using 220 nm filter paper.

(D) FE-SEM Analysis

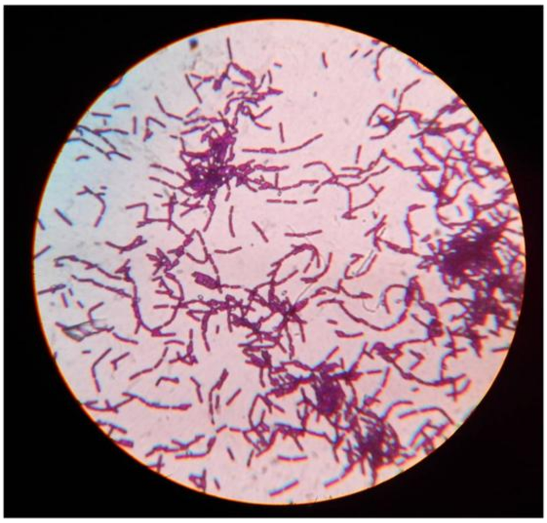
The morphology of nanoparticles was studied using scanning electron microscopy, taking $AgNO_3$ treated (at MIC value) bacterial samples from the stationary phase of growth kinetics. For FE-SEM slide preparation, 1.5ml of overnight culture was taken.The centrifugation was done at 5000 rpm for 5min.The pellets were collected and washed twice with PBS. Again centrifugation was done. Then the pellets were collected by centrifugation at 5000rpm for 5 min. pelletes were resuspended in PBS. The glutaraldehyde (2.5%) was flooded over the glass slide, and kept for overnight (15hrs) incubation. After 15hrs 1% of tannic acid was flooded over the slides. The slides were kept for 5min. The slides were washed with distilled water and dehydrated with ethanol series (30%,50%,70%,90% and 100%), kept for drying. The samples were characterized using FE-SEM.

(E) ATR-FTIR ANALYSIS

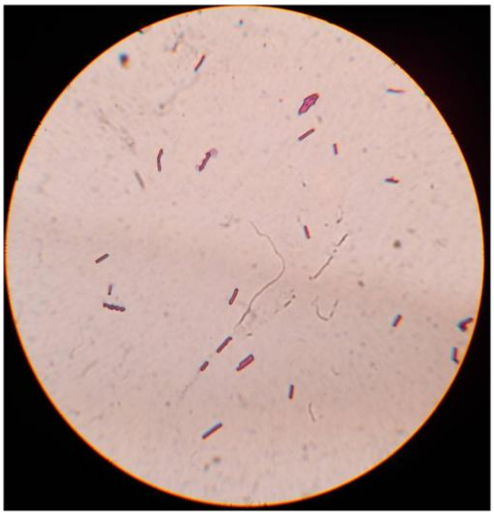
The samples were then characterized by ATR-FTIR. It gives information about the bond level vibration of samples, hence an important technique for identification and characterization of nanoparticles. The basic composition of the compound can be determined by spectral location of their IR absorption.

4.RESULT AND DISCUSSION

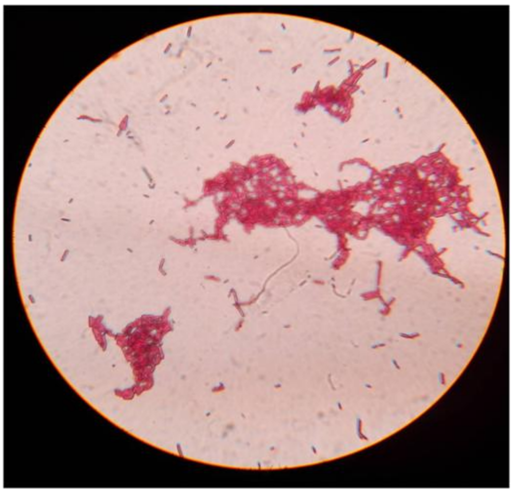
4.1. Gram staining



(a)



(b)

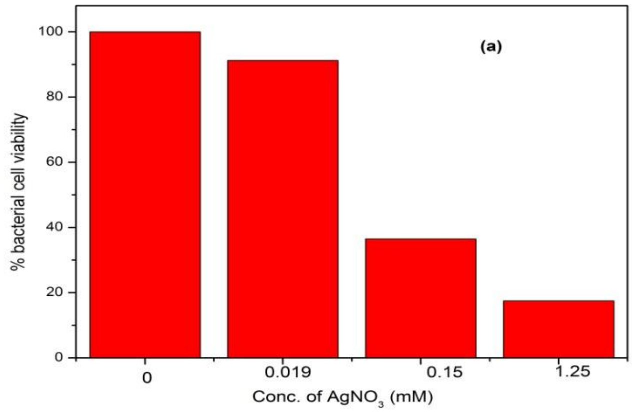


(c)

FIG:2:- The bacteria strain: (a) Gram +ve , (b) Gram +ve, (c) Gram -ve bacteria

Fig 2(a), (b), and (c) show the result of Gram staining .From the three bacteria we found first and second are gram +ve and third one as Gram-ve bacteria.The first and second bacteria are violet colour and third one is pinkish in colour, so earlier two are Gram positive and later one is Gram negative according to the principles of Gram staining. Here, we haven chosen first two bacteria for silver nanoparticle synthesis as they are Gram positive.

4.2. Determination of $AgNO_3$ MIC



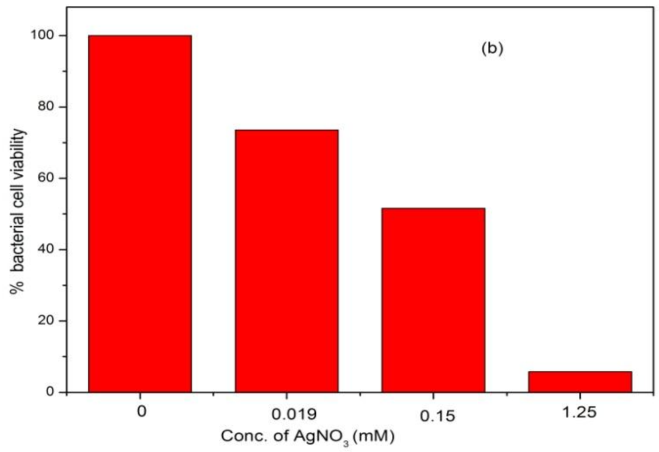


Fig:3 – Bacteria cell viability at different concentrations of $AgNO_3$:(a) bacteria 1 (b) bacteria 2.

Fig.3a and 3b show the viability of both bacteria treated with different concentrations of $AgNO_3$.analyzed by measuring CFU. From this results it is confirmed that 0.15 mM conc. of $AgNO_3$ is capable of significantly inhibiting the bacterial growth, so we have chosen 0.15 mM as MIC value for synthesis of nanoparticles.

4.4: UV-VISIBLE Spectroscopy

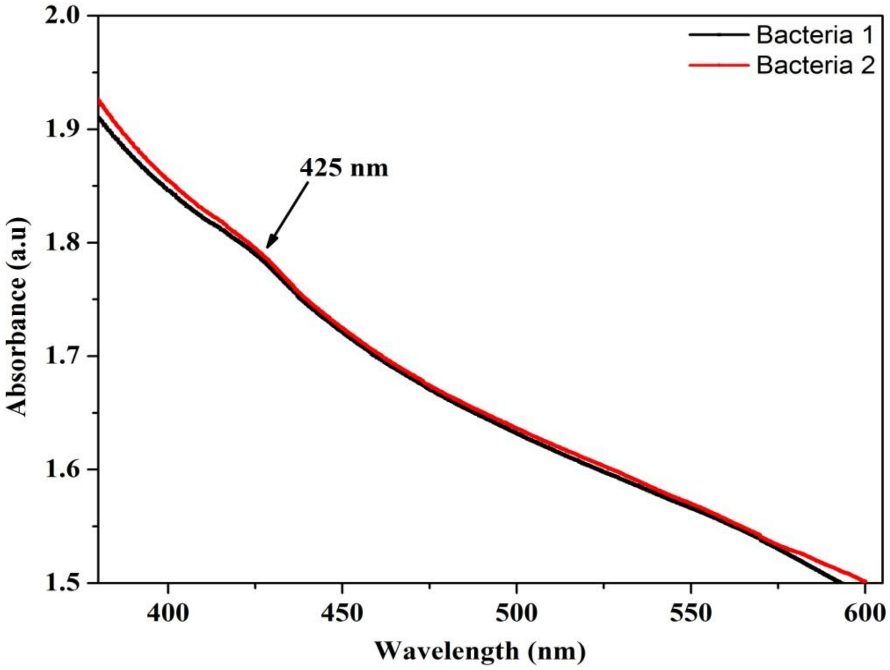
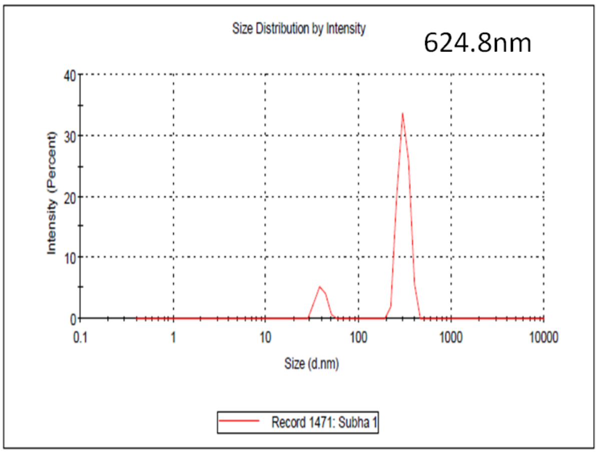


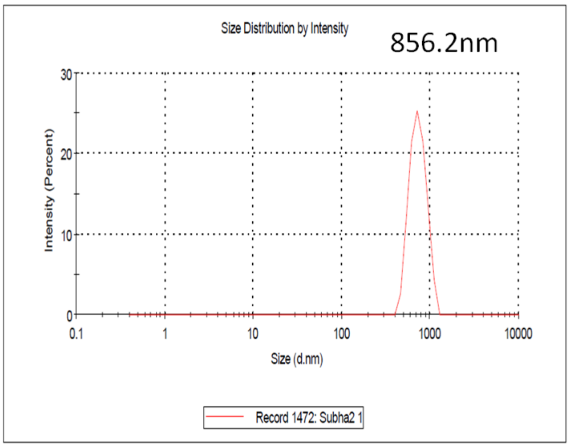
Fig:4:- UV- Visible spectroscopic analysis of synthesized nanoparticles

The sample containing the silver nanoparticles was characterized using UV- Visible spectroscope. From the fig 4, we found the absorance peak to be at 425nm, which is the characteristic property of silver nanoparticles as suggested by different literatures (Pal, Tak et al. 2007; Mude, Ingle et al. 2009).

4.5:-DLS Analysis

To determine the particle size we perform dynamic light scattering technique. Here the size of the particle is responsible for light scattering. Particle with greater radius diffract the light less than that of a smaller radius particle. The fig 5 shows the DLS analysis of silver nanoparticles. The average particle size determined by DLS was found to be 624 nm and 856 nm for bacteria 1 and bacteria 2 respectively.





(a) (b)

Fig:5:-DLS analysis of synthesized silver nanoparticles

4.6: ZETA POTENTIAL analysis

Table 1:-zeta potential analysis of sample

Sample	Net charge
Sample1	-16.9 mV
Sample2	-13.0 mV

Zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. Table below shows the zeta potential value of silver nanoparticle. The zeta potential value of sample 1 is -16.9 mv and sample 2 is -13.0 mv. It indicates that the surface of the synthesized silver nanoparticle has negative charge.

4.7:FTIR Analysis

Since every molecule has the property to absorb the light in IR region and this absorption makes the vibration of the bonds present in the molecule. The peaks at 562 and 598 cm^{-1} for AgNPs synthesized from bacteria 1 and 2 confirm the presence of AgNPs in the samples. The peaks at 1657 and 1633 cm^{-1} are due to amide I vibrations of proteins and 1530 and 1538 cm^{-1} are due to amide II vibrations of proteins. So it is concluded that proteins play important role for synthesis of AgNPs.

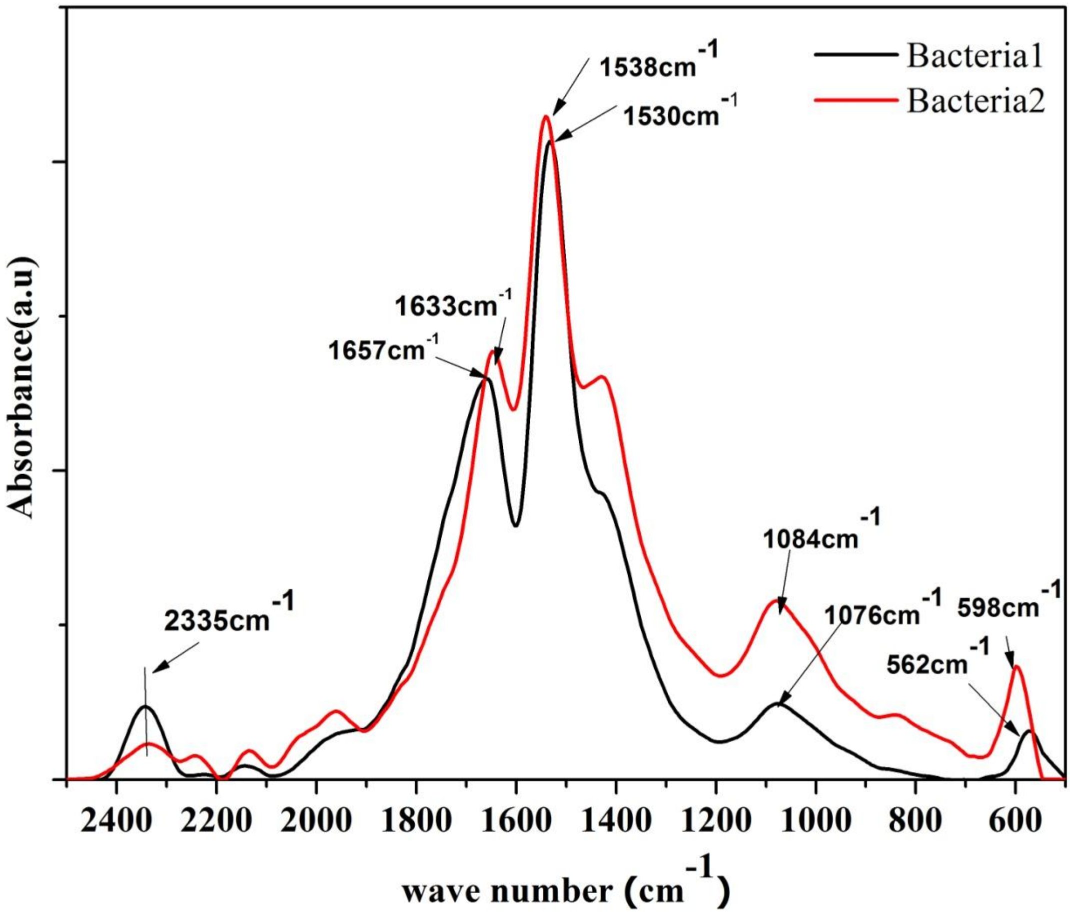
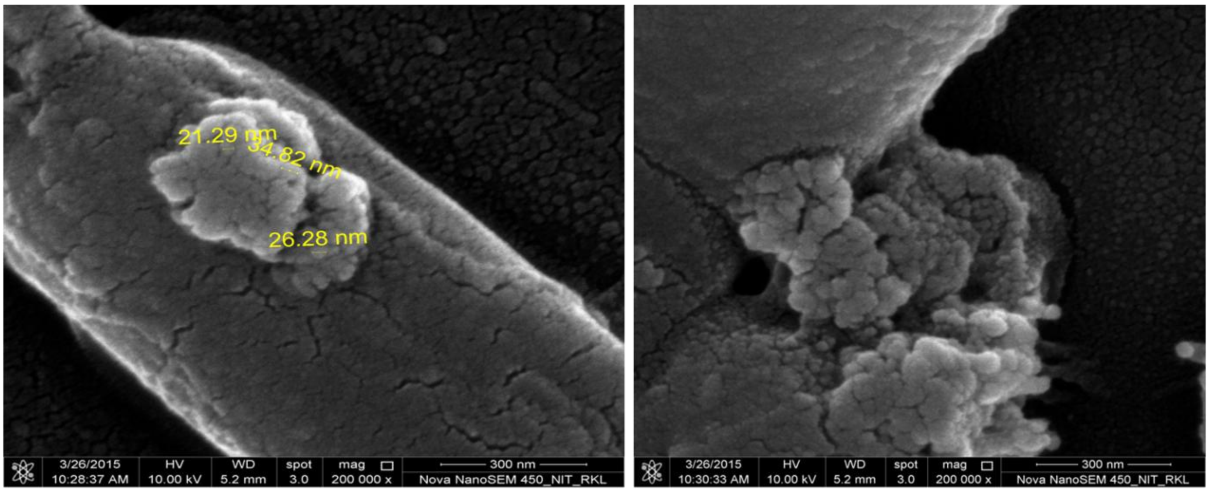


Fig:6- FTIR Analysis of synthesized silver nanoparticle

4.8: FE-SEM Analysis

From the FE-SEM analysis, we conclude that the synthesized nanoparticles are of spherical form with different sizes ranging from 20 nm to 35nm.



(a) (b)

Fig:7:-FE-SEM analysis of nanoparticles

5.CONCLUSION

From this work ,it is concluded that the bacteria present in coal mines are capable of synthesizing silver nanoparticles. The synthesized nanoparticles show similar characteristic properties as silver nanoparticles synthesized from other sources. Moreover, the proteins present in the samples are found to be responsible for synthesis of nanoparticles as suggested from ATR-FTIR study.

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